Journal of Shellfish Research, Vol. 27, No. 2, 323–336, 2008.

PARENTAL RELATEDNESS AND SURVIVAL OF PACIFIC OYSTERS FROM A NATURALIZED POPULATION

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ABSTRACT Inbreeding has profound implications in fields ranging from evolutionary biology to medicine. Most cultured aquatic species are only partially domesticated and highly fecund and are, therefore, expected to have higher genetic load and more severe inbreeding depression than species with lower fecundity and/or longer histories of domestication. Marine bivalves such as oysters are extreme in this regard, and previous studies have demonstrated that self-fertilization, brother/sister matings, and cousin/cousin matings in Pacific oysters (*Crassostrea gigas*) reduce growth and survival. It is unclear, however, whether these effects can be extrapolated to lower levels of consanguinity such as those expected in natural populations or the founders used to initiate cultured strains or selective breeding efforts. To address this without the need for extensive pedigree information, we studied inbreeding in a naturalized population of Pacific oysters using molecular marker-based estimates of parental relatedness calculated from multilocus microsatellite genotypes. We produced 34 full-sib families using randomly mated parents collected from a naturalized population in Dabob Bay (Washington, USA) and planted them in intertidal and subtidal conditions in Dabob Bay and Yaquina Bay (Oregon, USA). Using 16 microsatellite loci, we estimated the degree of inbreeding of each pair's progeny in three ways: (1) identity (***I***) or the expected homozygosity of the progeny based on their parents' genotypes estimated using the IDENTIX program, (2) a moment-based estimator of the pair-wise coefficient of relatedness (***W***) of each parental pair estimated using the MER program of Wang, and (3) a modified estimator of the pair-wise coefficient of relatedness (***K***) designed specifically to accommodate null alleles, which were common in the Dabob Bay population and estimated using the ML-RELATE by Kalinowski. Using a composite analysis of variance approach to partition the total among-family variation into components attributable to inbreeding and all other family-level effects, we found statistically significant negative relationships between all three estimators of inbreeding and the survival of their progeny when we analyzed all location/exposure combinations simultaneously and in 7 of the 12 separate tests examining each location/exposure combination separately. However, for 4 of the 5 nonsignificant tests, $P < 0.07$ using ***K*** estimator of relatedness, which makes the potentially unreasonable assumption that all parents have inbreeding coefficients (F) of zero. Further, these relationships were strongest at the site with the highest mortality, indicating that inbreeding depression may be more severe in a more stressful environment. Finally, we briefly outline potential strategies for using molecular marker-based estimates of relatedness to improve the genetic composition of new founder populations, incorporation of relatedness information in selective breeding efforts, and minimizing inbreeding effects in established cultured oyster populations.

KEY WORDS: *Crassostrea gigas*, genetics, relatedness, heterosis, inbreeding depression, selective breeding

INTRODUCTION

Mating among relatives and its consequences can have profound effects on mating systems, mate-choice behavior, dispersal, kin selection, population structure, the maintenance of genetic variation, and the genetic architecture of quantitative traits. Typically, inbreeding is deleterious, and in theory inbreeding depression can be caused by negative effects of deleterious recessive alleles when homozygous or the diminution of positive heterotic effects (Lynch & Walsh 1998). These two mechanisms are not mutually exclusive, but the former is generally believed to be more common (Charlesworth & Charlesworth 1999). Most cultured aquatic species are only a few generations removed from natural stocks, and their high fecundity makes it possible to quickly generate large cultured populations from a small number of founding parents, predisposing cultured populations to small effective population sizes and high rates of inbreeding. Furthermore, whereas long-domesticated species have had ample opportunity to purge deleterious alleles, large wild populations and semidomesticated populations recently derived from them are much more likely to carry a high genetic load of deleterious alleles and thus suffer from inbreeding depression.

Conceptually, the simplest approach to studying the effects of within-population inbreeding uses pedigree records of natural or controlled matings to unambiguously determine individual inbreeding coefficients (F) relative to some reference generation and to ask if there is a relationship between F and fitness or its component traits. In practice, however, this approach can be difficult to apply. Unless long-term pedigree records are available, only recent inbreeding can be examined. In the context of aquaculture, this can be particularly problematic because undetected family structure in wild populations combined with the low number of founder individuals necessary to initiate cultured populations can result in small founder populations containing unrecognized relatives. Under these circumstances, even strict avoidance of subsequent inbreeding could result in significant inbreeding depression caused by consanguineous matings among unrecognized relatives.

An attractive alternative when pedigree information is unavailable is to study within-population inbreeding using molecular markers. These data are relatively simple to acquire

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compared with long-term pedigrees, and since the development of allozyme markers, biologists have been devoting considerable effort to investigations of the associations between marker-based estimates of inbreeding and fitness-related characters. Traditionally, multilocus heterozygosity (MLH) has been used as a proxy for individual-level inbreeding, and a number of studies have found significant correlations between multilocus heterozygosity and fitness in a wide range of taxa (for reviews see Avise 1994, Britten 1997, David 1998, Hansson & Westerberg 2002, Mitton 1993, Roff 1997). However, this estimator ignores the crucial difference between alleles identical by state and identical by descent and therefore provides at best rather crudely approximates F. A recently-developed variation on MLH is so-called ''internal relatedness.'' This measure weights locus-specific heterozygosity using allele frequencies on the premise that shared rare alleles are more informative indicators of inbreeding than shared common alleles (Amos et al. 2001). Another individual-level estimator specific to microsatellite DNA markers quantifies the degree of divergence between the two alleles carried by a single individual at a single locus as the squared difference in the number of tandem repeats (d^2). Averaged over a number of loci, this measure, like multilocus heterozygosity, estimates the genome-wide degree of similarity of the two alleles carried by a diploid individual (Coulson et al. 1998) assuming stepwise mutation. This estimator has, however, been criticized as not substantially better than heterozygosity, and in some cases worse (Goudet & Keller 2002, Hedrick et al. 2001, Tsitrone et al. 2001).

A slightly different approach is to use multilocus marker genotypes to estimate parental relatedness directly for known mating pairs rather than to indirectly compare alleles within individuals of unknown parentage. Relatedness estimators fall into two broad groups. The first uses population-level allele frequency data to assign pairs of multilocus genotypes relationship categories such as full-sibs, half-sibs, parent-offspring or unrelated using maximum likelihood (Fernandez & Toro 2006, Mousseau et al. 1998, Smith et al. 2001, Thomas 2002, Wagner et al. 2006). The second uses moment-based estimators of continuously-distributed relatedness coefficients between pairs of individuals by estimating the genome-wide probability that alleles are identical by descent (Kalinowski et al. 2006, Li et al. 1993, Lynch 1988, Queller & Goodnight 1989, Wang 2002). The first group is most useful when only a few categories of relationship are possible whereas the second is more appropriate when a wide variety of relationships are expected. Few studies have addressed inbreeding by directly estimating the pair-wise relatedness of parents and its relationship to the performance of their progeny, though the approach has been applied to birds (Cohen & Dearborn 2004, Hansson 2004), termites, (DeHeer & Vargo 2006), and plants (Souto et al. 2002).

Whereas all aquaculture species are more prone to inbreeding in the hatchery than in the wild and are unlikely to have purged their genetic load than long-domesticated species, bivalve molluscs may be the worst-case scenario. In species with extremely high fecundity, high dispersal, unpredictable recruitment, and sessile adults, recruitment is a ''lottery'' in which the low probability of any single ticket winning makes purchasing many differently-numbered tickets the best strategy for success (Williams 1975). As a consequence, natural selection favors offspring variability and thus the evolution of outcrossing mating systems and high mutation rates in high fecundity species. Because the vast majority of mutations are at least mildly deleterious (Lynch et al. 1999), these species are also expected to carry a heavier genetic load of deleterious recessive alleles than low-fecundity species with limited dispersal. Consistent with this, the genetic load of the Pacific oyster has been estimated to be equivalent to about 15–20 lethal mutations per oyster or approximately five times the genetic load in humans or fruit flies (Launey & Hedgecock 2001, Bucklin 2002).

Recent studies of inbreeding in bivalves all compare outcrossed progeny to highly consanguineous matings such as selfed hermaphrodites (Bucklin 2002, Hedgecock et al. 1995, Launey & Hedgecock 2001, McGoldrick & Hedgecock 1997) or brother/sister matings (Beattie et al. 1987, Bierne et al. 1998, Imai & Sakai 1961, Lannan 1980, Longwell & Stiles 1973, Mallet & Haley 1983). To our knowledge, only one study of inbreeding in cupped oysters and one study in flat oysters (genus *Ostrea*) have studied inbreeding less severe than matings among full-sibs. Evans et al. (2004), evaluated the growth and survival of *C. gigas* progeny with three levels of inbreeding ($F = 0$, 0.0625 or 0.203) and found a linear relationship. Naciri-Graven et al. (2000) studied inbreeding depression in the European flat oyster, (*O. edulis*) by creating single-pair crosses within three stocks selected for disease resistance. In two of these stocks, they reconstructed the single-generation pedigrees of the parents using molecular markers to assign them to the grand parents of the progeny they evaluated, and in the third population they calculated the relatedness between parental pairs using Queller and Goodnight's (1989) relatedness estimator because parentage assignment was too difficult. Their parentage assignments produced estimates of the inbreeding coefficient of progeny ranging from 0 to 0.25, and their marker-based estimates of relatedness ranged from 0 to 0.9. They found substantial inbreeding depression for growth using pedigree analyses, and a nonsignificant negative trend between marker-based parental relatedness and growth.

Recently, large number of highly polymorphic microsatellite markers have been developed for *C. gigas*, providing the requisite molecular technology to access genetic-level information directly (Huvet et al. 2000, Li et al. 2003, Magoulas et al. 1998, McGoldrick et al. 2000, Yamtich et al. 2005). In this study, we use these relatively new molecular tools to extend our knowledge of inbreeding in oysters to lower levels of consanguinity than previous studies: randomly-mated pairs sampled from a natural population. Understanding inbreeding at this level is important because these natural populations are the source from which the founding populations for domestication and selective breeding are derived, and the genetic relationships among the specific individuals removed from these populations for culture can, at least potentially, have profound impacts on the genetic composition of cultured populations derived from them, including their levels of genetic variation, potential for genetic improvement, and the genetic load they carry. To evaluate the potential consequences of establishing a closed breeding population from a modest number of individuals taken from a wild population, we collected parents directly from a wild oyster population and used three different molecular marker-based estimates of the relatedness among parents to determine the levels of inbreeding of their progeny. We also divided each experimental family and grew them at several field sites

to test whether these effects on survival were consistent across environments.

MATERIALS AND METHODS

Collection, Spawning and Nursery Protocols

We collected 300 adult *C. gigas* from Dabob Bay, WA, USA (47.8°N, 122.87°W) and transported them to the Hatfield Marine Science Center (HMSC), Newport, OR, USA (44.6°N, 124.1°W), in January 2002. These potential parents were held in 18°C sand-filtered seawater and fed a mixture of *Isochrysis galbana* (Iso) and *Chaetoceros calcitrans* (Cc) at a concentration of approximately 50,000–80,000 cells mL^{-1} until ready to spawn. In April 2002, we strip spawned 68 individuals on the same day as per Langdon et al. (2003) and created 34 full-sib families. Fertilized eggs were allowed to develop into veliger larvae (D-larvae) for 24 h in cross-specific 20-L containers filled with 0.2 µm-filtered seawater at 25°C.

We then stocked D-larvae from each cross into 60-L larval culture containers at a concentration of 10 larvae mL^{-1}. We fed the larvae daily with a mixture of Iso and Cc at concentrations ranging from 30,000–80,000 cells mL^{-1}, depending on age (Breese & Malouf 1975). We drained all of the larval tanks, cleaned them with hot fresh water, and refilled them with 0.2-µm filtered seawater at 25°C twice per week. During the first week, larvae were retained on 37-µm sieves, and for the second on week 80-µm sieves. During the third week, we poured the larval cultures through stacked 243-µm and 80-µm sieves. All larvae retained on the 243-µm sieve we then exposed to 2×10^{-4} M epinephrine to induce metamorphosis (Coon et al. 1986). No data were collected on larval growth or survival.

We transferred successfully metamorphosed juveniles (spat) to culture-specific 15-cm diameter upwelling silos held in a semirecirculating system (approximately 6 exchanges day^{-1} of UV-irradiated, 1-µm filtered seawater at 25°C). Once all larvae had metamorphosed, we randomly reduced the number of spat per silo to 10,000. Spat were allowed to grow until they were retained on a 1.4 mm sieve, then transferred to a larger upwelling system (28-cm diameter silos). These larger upwellers were supplied with 18°C, 1-µm filtered seawater and fed a mixure of Iso and Cc at a final concentration of approximately 50,000–80,000 cells mL^{-1}. Once all animals were transferred from the 15-cm upwellers, we randomly reduced the number of oysters per 28-cm silo to 5,000. Oysters were then allowed to grow until retained on a 6.4-mm sieve, before being transferred to culture-specific spat bags (2 mm mesh) held in storage tanks receiving ambient 1-µm filtered seawater (mean 12.4°C; range 9.9°C–18.4°C) and batch-fed to a final concentration of approximately 80,000–100,000 cell mL^{-1} of a Cc/Iso mixture twice per week until all families were ready for planting into the field.

Twelve replicate bags of 40 oysters from each family were weighed for each of the two subtidal sites (utilizing 10-tier, 0.51-m diameter lantern nets; 5 mm mesh) and 12 replicate bags of 60 oysters from each family were weighed for each of the two intertidal sites (utilizing rectangular 0.53 m × 0.81 m growout bags; 7 mm mesh). Regardless of culture method, all oysters were first stocked into 0.3 m × 0.3 m sleeves (2 mm mesh) sleeves. These sleeves were then inserted into either lantern nets compartments or growout bags.

Field Trials

Four growout environments were examined in this study: 1. intertidal on-bottom culture in Yaquina Bay, OR; 2. subtidal suspended culture in Yaquina Bay, OR; 3. intertidal on-bottom culture in Dabob Bay, WA; 4. subtidal suspended culture in Dabob Bay, WA (Fig. 1). These four environments were intended to represent four very dissimilar oyster-growing environments encountered in the Pacific Northwest (Quayle 1988). Yaquina Bay is an estuarine environment subject to tidal and seasonal fluctuations in salinity, which can range from 0‰ caused by freshwater runoff in the winter to 35‰ during high tide in the summer. Conversely, Dabob Bay is a deep embayment off Hood Canal in Puget Sound. This environment is characterized by constant and high salinity, decreasing only slightly during winter rain-events (Warner et al. 2001). Data loggers (F. Smith; Northwest Research Associates, Seattle, WA) were positioned at a depth of approximately 1 m at the subtidal sites and at a tidal height of approximately +0.3 m MLLW at the intertidal sites and recorded temperature at 2 h intervals and salinity at 0.5 h intervals.

Each environment was partitioned into three blocks, accounting for either intertidal aerial exposure or subtidal depth in the lantern nets. Each family was represented by up to four replicates per block; however, due to variable survival in the nursery, each family had, on average 3 replicate bags per block. In February of 2003 (day 192 in the field) oysters were

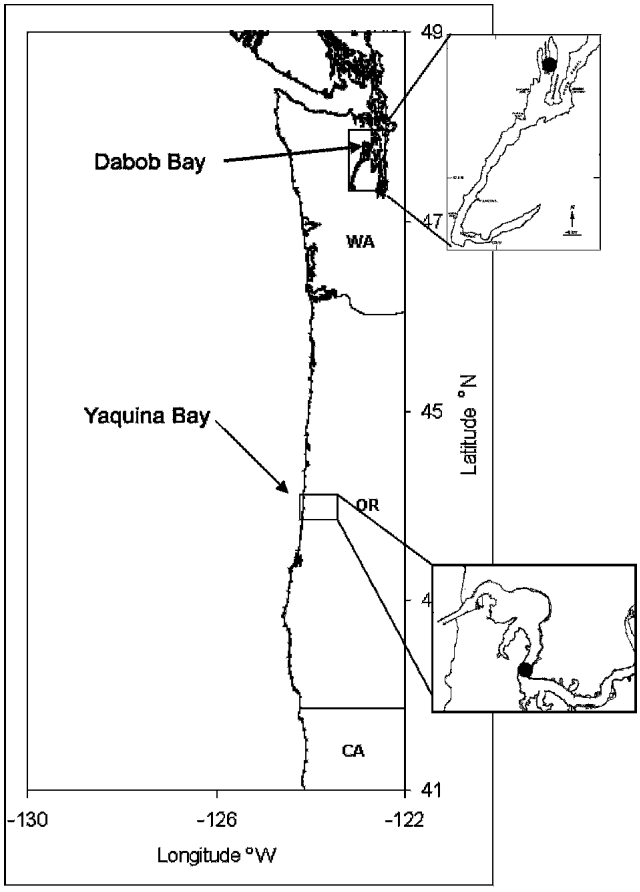

Figure 1. Map of the coasts of Washington and Oregon USA, showing the locations of the field sites.

removed from the 2-mm mesh sleeves, and cleaned of all biotic and abiotic fouling. The surviving oysters from each growout unit were then counted and collectively weighed. The collected data provided replicated estimates of average family bag weight (kg replicate^{-1}) and survival (%) in the field. Average individual oyster weight (weight of the body and shell) per replicate was calculated by dividing total bag weight by the number of live oysters in each growing unit.

We then restocked the surviving oysters directly into either intertidal growout bags (7 mm mesh) or subtidal lantern nets (5 mm mesh). At the end of the first summer growing season (August 2003; day 370), we again cleaned the growout equipment and the oysters, and collected the same data. Likewise, we harvested all of the oysters at the end of the second growing season (June 2004; day 664 at Dabob Bay and day 697 in Yaquina Bay), and collected all the same data.

Microsatellite Genotyping

After spawning, we individually froze the bodies of all of the parental oysters in separate Whirl-Pak bags at –80°C for subsequent genotyping. From these frozen bodies, we later removed small pieces of mantle tissue (approx 1 mm^3) and extracted DNA using the Qiagen DNEasy 96-well kit according to the manufacturer's instructions. We then amplified 16 microsatellite loci in 5-μL reactions in 384 well plates using the locus-specific annealing temperatures and magnesium concentrations indicated in Table 1. In some cases, these conditions were reoptimized in our laboratory and differed from the conditions published by the researchers who originally developed the markers. These reactions were run on an MJ Research Tetrad thermocycler according to the following program: 94°C for 5 min, followed by 30–40 cycles of 94°C for 30 sec, a locus-specific annealing temperature for 30 sec, and 72°C for 45 sec. A final extension of 72°C for 30 min completed the PCR. We visualized the lengths of the fragments produced on an Applied Biosystems 3730 XL DNA analyzer and identified specific alleles at each locus using the GeneMapper software package version 3.5 (Applied Biosystems Inc., Foster City, CA).

We used the program FSTAT (Goudet 1995) to calculate F_{is} (Weir & Cockerham 1984) for each locus and across all loci. F_{is} estimates within-population deviations of genotypic frequencies from expectations based on population-level allele frequencies and Hardy Weinberg equilibrium (HWE). In the absence of population substructure and immigration, homozygote excesses ($F_{is} > 0$) indicate inbreeding. However, genotyping errors, especially null alleles can also produce apparent homozygote excess. To examine this possibility, we used the program MICROCHECKER (Van Oosterhout et al. 2004) to examine our genotypic data for the presence of null alleles and estimate their frequency. MICROCHECKER does not produce rigorous statistical tests for the presence of null alleles, but by summarizing the distribution of homozygote excess within and among loci, provides an indication of the underlying genetic mechanisms. An even distribution of homozygote excess at all allele sizes within a locus, combined with inconsistency among loci in deviations from expectations under HWE strongly implies the presence of null alleles. Other PCR artifacts such as large allele dropout and stuttering are expected to vary systematically with allele size, and alternative population-level explanations such as inbreeding, assortative mating, and population structure (Wahlund effects) are expected to produce consistent patterns at all loci.

Relatedness Estimation

We calculated three different molecular marker-based estimators to characterize the level of inbreeding expected for each pair of parents. The first, Identity (***I***) is simply the expected proportion of homozygous loci in the offspring of a particular pair, which we estimated using the program IDENTIX (Belkhir et al. 2002). The second was Wang's maximum-likelihood based

TABLE 1.

PCR conditions, number of alleles detected, F_{is} and significance tests (* = $P < 0.05$ after Bonferonni correction, ns = not significant), estimated frequency of null alleles when their presence is indicated, and reference to the original publication describing the development of each microsatellite marker.

Locus	Anneal Temp (°C)	[MgCl^2] (mM)	# Alleles	F_{is} (P)	Estimated Null Frequency	Citation
ucdCg003	50	1.0	45	0.183 (*)	0.089	McGoldrick et al. 2000
um2CgL10	54	1.0	37	0.055 ns	—	Huvet et al. 2000
ucdCg018	52	1.5	20	0.447 (*)	0.215	McGoldrick et al. 2000
ucdCg021	50	1.5	40	0.176 (*)	0.085	McGoldrick et al. 2000
imbCg049	52	1.5	29	0.152 (*)	0.073	Magoulas et al. 1998
ucdCg126	56	1.5	29	0.425 (*)	0.209	Li et al. 2003
ucdCg171	54	1.5	13	0.202 (ns)	0.100	Li et al. 2003
ucdCg172	58	1.5	5	0.084 (ns)	—	Li et al. 2003
ucdCg119	50	1.5	47	0.412 (*)	0.202	Li et al. 2003
ucdCg120	50	2.0	12	0.032 (ns)	—	Li et al. 2003
ucdCg160	56	1.0	33	0.492 (*)	0.242	Li et al. 2003
ucdCg195	60	2.0	13	0.239 (*)	0.115	Li et al. 2003
ucdCg197	52	1.5	59	0.208 (*)	0.101	Li et al. 2003
ucdCg199	54	1.5	8	0.656 (*)	0.301	Li et al. 2003
ucdCg200	56	1.5	17	0.249 (*)	0.120	Li et al. 2003
ucdCg202	48	2.0	26	0.559 (*)	0.273	Li et al. 2003
Loci Averaged			27.1	0.285 (*)	0.163	

moment estimator of relatedness (***W***), which we estimated using the program MER v3 (Wang 2002). In this analysis, the 68 parental genotypes were used to estimate the population-level allele frequencies. The third relatedness estimator was Kalinowski's maximum likelihood estimator of relatedness (***K***), which we calculated using the program ML-Relate (Kalinowski et al. 2006). Again, the allele frequencies in the sample of parents were used to represent the population from which they were collected.

Whereas similar in concept to Wang's estimator, the ML-Relate program differs from MER in several important respects (Kalinowski et al. 2006). First, ML-Relate can estimate the frequency of null alleles at each of the loci and incorporate extra terms in the likelihood function to take into account the possibility that apparent homozygotes are, in fact heterozygotes with one undetectable null allele. It is also worth noting that these modifications do not make any assumptions about the number of null alleles in the population. Second, rather than using a moment estimator, the program uses a search algorithm to explore the allowable parameter space for the highest likelihood value of the underlying parameters given the data. The allowable parameter space is defined under the assumption that the two individuals being evaluated are not themselves inbred, and as a consequence, includes boundary conditions that disallow negative estimates of pairwise relatedness. The consequences of violating this assumption have not been explored (Kalinowski et al. 2006). This is in contrast with ***W*** for which negative estimates of relatedness indicate that the pair of parental genotypes under consideration have fewer alleles identical by descent than expected by chance (Hardy 2003) and that their progeny are less inbred than expected in the population under study regardless of its value of F_{is}. In effect, using ***K*** as we have here to measure of the level of inbreeding in their progeny amounts to assuming that $F_{is} = 0$ in the source population because the program assumes all parents have $F = 0$ and F_{is} is the population-level mean of individual-level inbreeding coefficients, F.

Statistical Analyses

All subsequent statistical analyses were conducted using SAS v.8 (SAS 2000). We first examined the correlations between the three measures of inbreeding using standard Pearson product-moment correlations.

We intended to examine the consequences of inbreeding on survival and growth as well as the genetic correlation between them. Estimating the genetic correlations among traits using families reared separately, however, can be problematic if differential survival among families results in environmental correlations driven by differences in density. Evans & Langdon (2006) addressed this possibility by examining the correlation between survival to the end of the first growing season (i.e., density at the beginning of the second growing season) and growth rate during the second growing season. They reasoned that if families with high stocking densities entering the second growing season exhibit slower than average second season growth, this should produce a negative correlation. In three separate experiments, they found two cases of no relationship and one significant positive correlation between density at the start of the second growing season and second season growth, suggesting that at the initial stocking densities used in their experiment, subsequent changes in density caused by differential survival have negligible effects on growth. Accordingly, we used similar initial stocking densities. However, to explicitly test for density effects in this experiment we estimated the correlations of family means between the number of surviving oysters at the beginning of a growth interval and three indicators of growth performance: (1) the average individual weight at the end of the interval (2) absolute growth during the interval, calculated as the average individual weight at the end of the interval minus the average individual weight at the beginning of the interval, and (3) proportional growth, calculated as the average individual weight at the end of the interval divided by the average individual weight at the beginning of the interval. All three measures were first calculated for individual growing units and then averaged within families. The first growing interval ran from February 2003 when the surviving animals were removed from the mesh sleeves, counted, weighed, and restocked directly into the larger growout units until August 2003 when they were again cleaned, counted, and weighed. The second interval ran from August 2003 until the oysters were harvested in June 2004. Because the two different exposure treatments used different growing units (bags *vs*. lantern nets) that were stocked with different numbers of animals (60 *vs*. 40), we performed separate correlation analyses for each location/exposure combination. These tests revealed significant density-mediated environmental effects on both size at age and growth (see Results), complicating the usual interpretation of differences in growth among families as broad sense genetic effects. As a consequence, subsequent analyses were performed for survival only.

Our main interests here are to (1) determine whether parental relatedness significantly covaries with survival in the field and (2) estimate the proportion of the total and among-family variance in survival attributable to parental relatedness. To evaluate this relationship, however, it is first necessary to account for the effects of other explainable sources of variation due, for example, to the effects of locations, tidal exposure, blocks, and preplant out effects such as size at planting and development time in the hatchery. Most of these are either design factors (location, exposure, blocks) or covariates measured on each growout unit at the time of planting (plantout weight) and thus can easily be accommodated by simply entering then into a single linear model and performing a standard analysis of covariance using Type III sums of squares that estimate the unique contribution of each factor in the model. However, hatchery developmental time and parental relatedness were measured at the level of entire families and cannot be included in the same model as a categorical family effect because they are essentially subcomponents of the among-family variance and are entirely subsumed by the categorical effect. Therefore, to remove the effects of hatchery development time and to explicitly test for effects of parental relatedness we used a composite analysis of variance approach to partition the overall among-family effects into three components, two single degree of freedom regression components for hatchery development time and parental relatedness and a multiple degree of freedom component representing all remaining among-family effects. This approach is analogous to testing for lack of fit or the adequacy of linear regression as discussed in most advanced textbooks (e.g., Neter et al. 1983, p. 128; Sokal & Rohlf 1981, p. 477). This partitioning was achieved using

three separate linear models (Table 2). In the first model, categorical family effects and all other sources of "nuisance" variation were included. In the second, the categorical family effect was replaced with the hatchery development time, and in the third, the categorical family effect was replaced with parental relatedness. The type III sums of squares from each of these models were then extracted and used to construct a composite ANOVA table that included four sums of squares: (1) a "corrected" SS(families) represented by the difference between the type III SS(Families) from the categorical model and the SS(hatchery development time) from the second model, 2) SS(Relatedness) taken from the third linear model (3) a SS(Difference) calculated by subtracting the SS(Relatedness) from the corrected SS(Families), and (4) the SS for error from the categorical among-families model representing the true unexplained variance.

From these SS and their corresponding degrees of freedom (df), we next calculated mean squares and F-ratios to test three separate hypotheses. The first F-ratio is MS(Family)/MS(error) and tests for a significant among-family component of variance, which not only subsumes the linear covariate effects, but also includes all other sources of among-family variance. It is important to note, however, that because this F-ratio uses MS(error) in the denominator, it treats families as a fixed effect rather than a random effect, which is atypical for quantitative genetic analyses. The F-ratio MS(Relatedness)/MS(error) provides a single df test for the linear covariate effect of parental relatedness on survival. Finally, the F-Ratio MS(Difference)/MS(error) tests for all of the additional contributions to the among-family variance not attributable to the relatedness covariate.

After examining normality plots of the residuals, we applied the angular transformation to the survival data (i.e., the proportion surviving to harvest within each growing bag). In addition, because of the boundary condition imposed by ML-Relate, this program estimated $\boldsymbol{K}$ as zero for many parental pairs. To accommodate this, we recoded these estimates using 0 to represent values estimated as 0 and 1 to represent any positive estimate. We then treated this relatedness estimator as a class variable in the analysis rather than as a continuously distributed covariate.

To examine whether the effect of inbreeding varied across environments, similar composite analyses were also performed on the transformed data for all four combinations of location and tidal exposure. We also used the ESTIMATE statement is SAS to obtain estimates of the regression slopes (or factor effects for $\boldsymbol{K}$) to determine the direction and magnitude of the effects of parental relatedness.

The strength of association for the among-family, relatedness, and difference effects, after removing nuisance variables was estimated as η^2_{alt} according Tabachnick and Fidell (1989) as

$$\eta^2_{alt} = \frac{SS_{effect}}{SS_{effect} + SS_{error}}$$

To quantify the strength of the relatedness covariate as a proportion of the overall among-family variation among, we also constructed another ratio, which we call η^2_{family} and calculated as

$$\eta^2_{family} = \frac{SS_{relatedness}}{SS_{family}}$$

RESULTS

Overall growth and survival data are shown in Table 3. The lowest overall survival occurred at the Dabob Bay intertidal site followed by the Yaquina Bay subtidal site. The Dabob subtidal and Yaquina Bay intertidal sites had the highest, and virtually identical rates of survival. At all sites, a majority of the mortality took place in the first year of deployment, but this is especially pronounced in the Dabob Bay intertidal site which experienced 43% mortality during this period whereas the other sites all suffered approximately 25% mortality during this phase of culture. Growth was also lowest at the Dabob intertidal site, and there was a strong effect of tidal exposure. The two intertidal sites showed greatly reduced growth

TABLE 2.

Factors entered into the linear models used to generate the composite analyses of variance.

All Sites Models	Site-Specific Models	Composite Analyses
Family Effects (Among, Hatchery Dev. Time, or Relatedness)	Family Effects (Among, Hatchery Dev. Time, or Relatedness)	Among Families (Corrected for Dev. Time)
Plantout Weight	Plantout Weight	Relatedness
Location	Block	Difference (by subtraction)
Exposure		Error (among family model)
Location * Exposure		
Family * Location		
Family * Exposure		
Family * Location*Exposure		
Plantout Weight*Location		
Plantout Weight*Exposure		
Plantout Weight*Location*Exposure		
Plantout Date*Location		
Plantout Date*Exposure		
Plantout Date*Location*Exposure		
Block(Location*Exposure)		
Error		

TABLE 3.

Average survival (%) and individual oyster weight (g) for the three sampling periods at each evaluation site.

Site	Survival (%)			Individual Weight (g)		
	Day 192	Day 370	Day 664/697	Day 192	Day 370	Day 664/697
Dabob Bay, Intertidal	57.0	48.5	47.2	1.9	14.1	46.0
Dabob Bay, Subtidal	73.2	64.8	62.8	6.4	46.3	104.7
Yaquina Bay, Intertidal	74.6	70.1	62.7	3.3	28.2	70.3
Yaquina Bay, Subtidal	75.2	59.1	52.2	2.1	56.7	106.9

compared with the two subtidal sites, in which growth was virtually identical.

The numbers of alleles detected at each of the microsatellite loci are presented in Table 1, and range from 5 to 59 with an average of about 27. Also presented in Table 1 are locus-specific estimates of F_{is} and tests of the hypothesis that observed F_{is} differs significantly from zero. Twelve of the 16 loci we used in this study showed a significant excess of homozygotes compared with expectations, with the exceptions of um2CgGL10, udCg120, ucdCg171, and ucdCg172. Furthermore, analysis with MICROCHECKER, supports the hypothesis that null alleles segregate in the Dabob Bay population at all but three of these loci um2CgGL10, ucdCg120, and ucdCg172 with estimates of the frequency of null alleles as ranging from as low as 5% to as high as about 27% (Table 1).

Figure 2 shows scatter plots of the relationships among the three measures of expected inbreeding used in this study and the results of pair wise correlation analyses. Several patterns are readily discernible. ***I***, the predicted proportions of homozygous loci in the offspring from each pair of parents is generally low, ranging from zero to about 0.2 with most values falling below 0.1. ***W***, Wang's moment estimator of relatedness, is also low overall, with a large number of negative values indicating that many parental pairs are less related than the expectations under random mating and reaches maximum of only about 0.05. Similarly, Kalinowski's estimator of relatedness, ***K***, is most frequently estimated as zero as a result of its boundary conditions and ranges only as high as about 0.08. All three measures of parental relatedness are significantly correlated with each other, but the strength of the relationship varies. ***I*** and ***W*** are highly correlated, but ***K*** is more weakly correlated with both ***I*** and ***W***, largely because of the large number of zero estimates imposed by the boundary conditions on its possible values.

The family means correlations between the stocking densities at the start of a growth interval and indicators of growth during that interval are presented in Table 4. The results are mixed, but there are significant ($P < 0.05$) negative family means correlations between stocking density and at least one indicator of growth performance at all 4 location/exposure combinations for both growth intervals.

Table 5 shows the composite analyses of variance for among-family variance and for each of the three relatedness measures when all locations and exposures are analyzed simultaneously along with estimates of the regression coefficients for relatedness effects, η^2_{alt}, and η^2_{family}. Table 6 shows

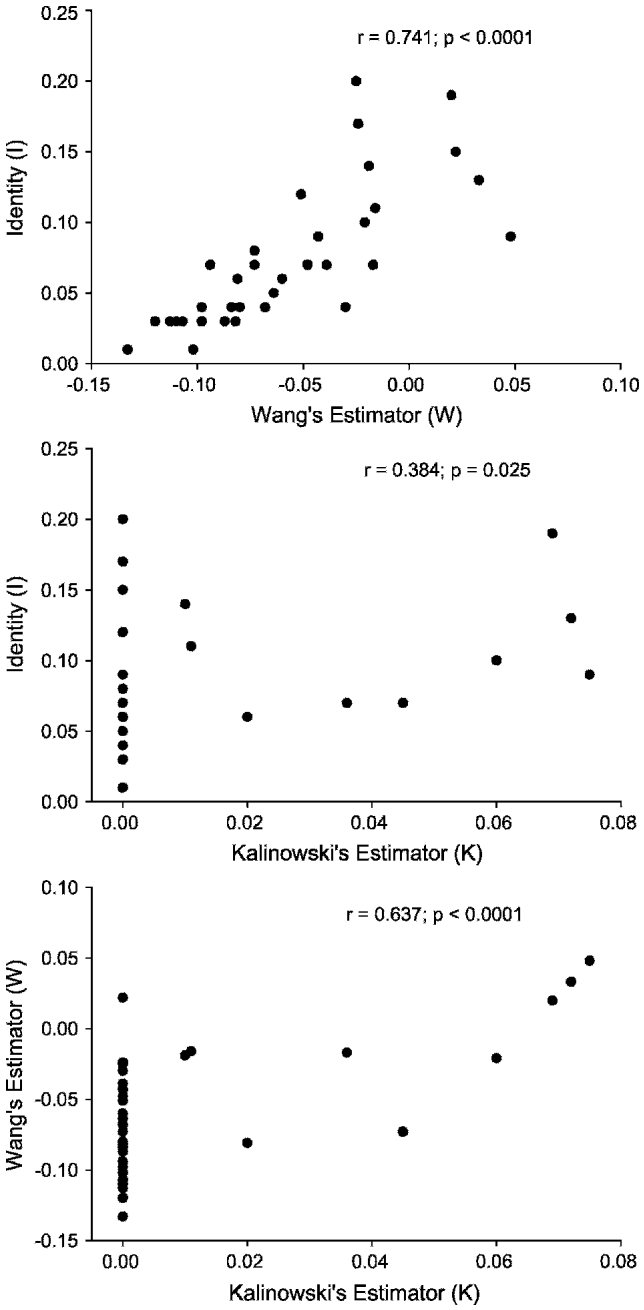

Figure 2. Scatter plots showing the relationship between the three estimators of parental relatedness.

the same hypothesis tests and parameter estimates when each location/exposure combination was analyzed separately. Looking first at the simultaneous analyses of all locations and exposures (Table 5), the among-family variance is, as expected, significant, and η^2_{alt} is approximately 0.5. In addition, all three relatedness estimators significantly covary with survival, and all regression coefficient estimates are negative, indicating that increasing parental relatedness is associated with reduced survival. η^2_{alt} for these analyses ranges from a low of 0.012 for ***K*** to a high of 0.07 for ***I*** with ***W*** intermediate at 0.044. Similarly, the proportion of the among-family variance attributable to parental relatedness (η^2_{family}) ranges from 0.011 for ***K*** to 0.069 for ***I*** with ***W*** again intermediate at 0.042.

TABLE 4.

Family means correlations between the stocking density at the beginning of each growth interval and three indicators of growth during that interval.

Site	Growth Interval	Individual Weight		Absolute Growth		Proportional Growth	
		R	P	R	P	R	P
Dabob	1	–0.506	0.008	0.559	0.003	–0.730	<0.001
Intertidal	2	–0.501	0.009	–0.482	0.013	0.112	0.586
Dabob	1	0.022	0.920	–0.035	0.871	–0.443	0.030
Subtidal	2	–0.314	0.135	–0.447	0.029	–0.549	0.006
Yaquina	1	–0.411	0.030	–0.444	0.018	–0.261	0.179
Intertidal	2	–0.631	<0.001	0.660	0.000	–0.309	0.110
Yaquina	1	–0.380	0.051	–0.368	0.059	0.100	0.618
Subtidal	2	–0.595	0.001	–0.596	0.001	–0.311	0.114

Site-by-site analyses (Table 6) are more complicated, at least partly owing to reduced statistical power given the reduction in sample size. In the Dabob Intertidal site, all estimates of the regression coefficients are still negative, with ***I*** and ***W*** still significant but the *P* value for ***K*** is 0.055. In the Dabob subtidal site, all coefficients are negative, but only ***I*** is significant with ***W*** and ***K*** having *P* values of 0.059 and 0.072 respectively. In the Yaquina intertidal, all coefficients are negative, with ***I*** and ***W*** significant and ***K*** with a *P* value of 0.067. Finally, at the Yaquina Bay subtidal site, all coefficients are negative; ***I*** and ***W*** are significant, but ***K*** has a decidedly nonsignificant *P* value of 0.52.

When all sites are analyzed simultaneously, the strength of association (η^2_{alt}) between the among-family variance and variance in survival is approximately 0.5 and η^2_{alt} values for the three parental relatedness estimators are quite small, ranging from about 0.01 for ***K*** to 0.07 for ***I*** (Fig. 3). Separate analyses for each location/exposure combination (Fig. 4) reveal that η^2_{alt} for overall among-family effects varies among locations, with stronger among-family effects in Yaquina Bay than in Dabob Bay, but the difference in η^2_{alt} between tidal and intertidal sites reverses. η^2_{alt} for the relatedness estimators is relatively stable among sites with the notable exception of ***K*** in the Yaquina subtidal site (Fig. 4). These two patterns interact such that η^2_{family} for the three relatedness estimators varies considerably among sites, with the highest values occurring in the Dabob intertidal and values for the other three sites being relatively stable, again with the notable exception of ***K*** in the Yaquina subtidal (Fig. 5).

DISCUSSION

Previous studies have used multilocus heterozygosity (MLH) as a proxy character for inbreeding in and found that MLH can be positively correlated with growth and survival in several species, including bivalves such as the Eastern oysters (Foltz et al. 1983, Singh & Zouros 1978, Zouros et al. 1980), *Spisula ovalis* (David et al. 1997) and blue mussels (Diehl & Koehn 1985, Koehn et al. 1988, Koehn & Gaffney 1984) in addition to a variety of plants and other animal species (David 1998, Hansson & Westerberg 2002, Houle 1989, Ledig 1986, Mitton 1993, Mitton 1997, Mitton & Grant 1984). However, the positive correlation between MLH and life-history traits, has been less apparent in single-pair crosses or crosses involving small numbers of parents in eastern and European oysters (Foltz & Chatry 1986, Gaffney & Scott 1984, Saavedra et al. 1996). Because cultured oyster populations fall somewhere between large natural populations and small experimental studies, it is unclear how to extend these previous results to aquaculture situations.

We found marked homozygote excesses in the Dabob Bay population of Pacific oysters, a common observation in bivalves (Hare et al. 1996 and references therein). Population substructure, inbreeding, population structure, and selection can all produce homozygote excesses, but so can genotyping artifacts such as nonamplifying null alleles and large allele dropout. Hedgecock et al. (2004) studied segregation patterns within families of *C. gigas*, and found that null alleles at microsatellite loci are common, and our data provide no evidence to the contrary. Estimates of F_{is} show significant positive departures from HWE and the distributions of homozygosity among loci and among allele sizes within loci suggest that these are likely to be attributable to null alleles.

Although the impacts of null alleles on sibship reconstruction and parentage assignment have been recently studied by a number of researchers, (Butler et al. 2004, Dakin & Avise 2004, Jones & Ardren 2003), we could only find one study that addressed how null alleles affect the estimation of relatedness coefficients (Wagner et al. 2006). These authors found that null alleles result in underestimation of the relatedness between individuals using standard estimators such as ***W*** and that the methods implemented in ML-Relate (Kalinowski et al. 2006) improve relatedness estimates more than removing loci that show evidence of null alleles from the data. To our knowledge, this is the only currently available method aside from eliminating loci for managing null allele issues in relatedness estimation, but this method also requires the potentially untenable

TABLE 5.

Composite analyses of variance for each estimator of relatedness with all location/exposure combinations examined simultaneously.

Source	SS	DF	MS	F	P	Estimate	η^2_{alt}	η^2_{family}
Among-Families	15.0787	26	0.5799	34.05	<0.0001		0.524	
I	1.0381	1	1.0381	60.94	<0.0001	–0.704	0.070	0.069
Difference (I)	14.0406	25	0.5616	32.97	<0.0001		0.506	0.931
W	0.6385	1	0.6385	37.48	<0.0001	–0.572	0.044	0.042
Difference (W)	14.4402	25	0.5776	33.91	<0.0001		0.513	0.958
K	0.1695	1	0.1695	9.95	0.0017	–0.011	0.012	0.011
Difference (K)	14.9092	25	0.5964	35.01	<0.0001		0.521	0.989
Error	13.7124	805	0.0170					

TABLE 6.

Composite analyses of variance with each location/exposure combination examined separately.

Source	SS	DF	MS	F	P	Estimate	η^2_{alt}	η^2_{family}
A. Dabob Bay Intertidal								
Among-Families	5.2891	24	0.2204	7.14	<0.0001		0.462	
I	0.5723	1	0.5723	18.55	<0.0001	–1.132	0.085	0.108
Difference (I)	4.7168	23	0.2051	6.65	<0.0001		0.433	0.892
W	0.3742	1	0.3742	12.13	0.0006	–0.890	0.057	0.071
Difference (W)	4.9150	23	0.2137	6.93	<0.0001		0.443	0.929
K	0.1148	1	0.1148	3.72	0.0551	–0.056	0.018	0.022
Difference (K)	5.1743	23	0.2250	7.29	<0.0001		0.456	0.978
Error	6.1693	200	0.0308					
B. Dabob Bay Subtidal								
Among-Families	3.5650	22	0.1620	12.50	<0.0001		0.600	
I	0.1252	1	0.1252	9.66	0.0022	–0.590	0.050	0.035
Difference (I)	3.4398	21	0.1638	12.63	<0.0001		0.592	0.965
W	0.0467	1	0.0467	3.60	0.0593	–0.319	0.019	0.013
Difference (W)	3.5183	21	0.1675	12.92	<0.0001		0.597	0.987
K	0.0423	1	0.0423	3.26	0.0727	–0.034	0.017	0.012
Difference (K)	3.5228	21	0.1678	12.94	<0.0001		0.597	0.988
Error	2.3731	183	0.0130					
C. Yaquina Bay Intertidal								
Among-Families	5.5748	26	0.2144	19.63	<0.0001		0.710	
I	0.1972	1	0.1972	18.05	<0.0001	–0.640	0.080	0.035
Difference (I)	5.3776	25	0.2151	19.69	<0.0001		0.703	0.965
W	0.1314	1	0.1314	12.03	0.0006	–0.524	0.055	0.024
Difference (W)	5.4434	25	0.2177	19.93	<0.0001		0.706	0.976
K	0.0369	1	0.0369	3.37	0.0676	–0.030	0.016	0.007
Difference (K)	5.5380	25	0.2215	20.28	<0.0001		0.709	0.993
Error	2.2722	208	0.0109					
D. Yaquina Bay Subtidal								
Among-Families	4.5618	25	0.1825	13.48	<0.0001		0.612	
I	0.2529	1	0.2529	18.68	<0.0001	–0.704	0.080	0.055
Difference (I)	4.3088	24	0.1795	13.26	<0.0001		0.598	0.945
W	0.1689	1	0.1689	12.47	0.0005	–0.572	0.055	0.037
Difference (W)	4.3929	24	0.1830	13.52	<0.0001		0.603	0.963
K	0.0056	1	0.0056	0.41	0.5213	–0.011	0.002	0.001
Difference (K)	4.5562	24	0.1898	14.02	<0.0001		0.611	0.999
Error	2.8978	214	0.0135					

assumption that the individuals being tested are not themselves inbred. Because none of these methods is free of complicating factors, we used three different relatedness estimators that we expected to be impacted differently by null alleles. As stated earlier, null alleles result in underestimates of parental relatedness using standard approaches such as ***W*** because null alleles distort population-level allele frequencies (Wagner et al. 2006). In contrast, null alleles would tend to result in inflated estimates of expected progeny homozygosity (***I***) and thus over-estimate the degree of inbreeding of progeny because this estimator makes no use of population-level allele frequencies. Finally, ***K*** applies corrections to improve the accuracy of estimation but this approach also makes the potentially unrealistic assumption that all the individuals whose relationships are being estimated have inbreeding coefficients of zero, which at the population level translates to no variance in individual level F and an F_{is} of zero for the population.

Whereas it may be surprising that parents collected directly from nature would be related at all, this finding is consistent with previous studies of the Dabob Bay population of Pacific oysters. Hedgecock et al. (1992) analyzed temporal variation in allozyme frequencies in the Dabob Bay oyster population for three different time intervals (1971–1972, 1972–1985; and 1971–1985) and estimated the effective population sizes (N_e) as 41.2, 337, and 502 respectively. Hedgecock (1994) argued that these low values are a consequence of high variance in reproductive success, a hypothesis also supported by subsequent analyses of larval genotypes using PCR-SSCP markers (Li & Hedgecock 1998). Thus, even small samples from this naturalized population are likely to include at least distant relatives because of low N_e. More importantly, however, the previous observation that this oyster population has low N_e makes it reasonable to expect that randomly collected pairs of parents taken from this population would produce a range of values for parental relatedness, and our goal was to test the hypothesis that this variance in parental relatedness contributes significantly to the well-documented among-family variance in survival observed in previous studies of inbreeding in Pacific oysters (Dégremont et al. 2007, Evans & Langdon 2006).

Encouragingly, the three different approaches used to estimate relatedness generally produced very similar results. The main exception is that the ***K*** estimator shows much weaker

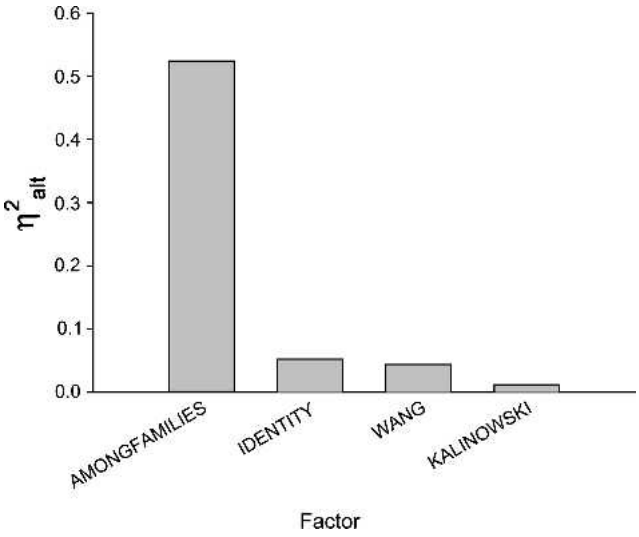

Figure 3. Strength of relatedness and among-family effects (η^2_{alt}) when all location/exposure combinations are combined into a single analysis.

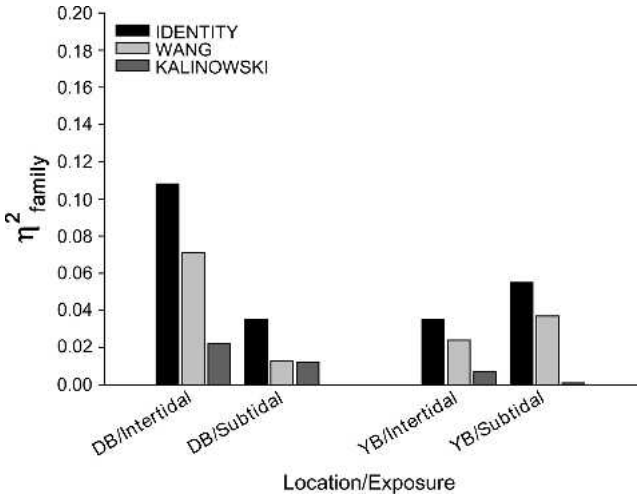

Figure 5. Strength of relatedness effect as a proportion of among-family variance (η^2_{family}) at each location/exposure combination. DB = Dabob Bay; YB = Yaquina Bay.

effects that are not statistically significant at some sites. It is difficult to determine, however, to what extent the differences between the result using ***K*** and the results using ***I*** or ***W*** are attributable to the assumption that the parents themselves are not inbred and that F_{is} in the Dabob population is zero. Whereas our estimates of F_{is} are complicated by the presence of null alleles, they and previous studies of the effective size of the Dabob Bay oyster population using allozyme markers certainly cast doubt upon the validity of the assumption that $F_{is} = 0$. Overall, however, our data support the hypothesis that even among randomly mated parents taken directly from a wild population, the resulting variance in parental relatedness and thus the levels of inbreeding in their progeny contributes to among-family variance in survival. The expected level of genome-wide homozygosity of progeny (***I***), Wang's moment estimator of pair wise relatedness (***W***), and Kalinowski's estimator of relatedness (***K***) all reveal negative covariance between the relatedness of parents and the survival of their progeny when all environments are analyzed simultaneously. Both broad-sense (Evans & Langdon 2006) and narrow-sense (Dégremont et al. 2007) genetic variation for survival in Pacific oysters is well documented and surprisingly large, and we expected inbreeding to explain at best a fraction of the among-family variation in survival with the remainder attributable to other additive and nonadditive sources of genetic variation among families. Consistent with these expectations, depending on the specific estimator used, variance in parental relatedness accounts for somewhere between 1% and 7% of the among-family variance in survival when all sites are analyzed simultaneously.

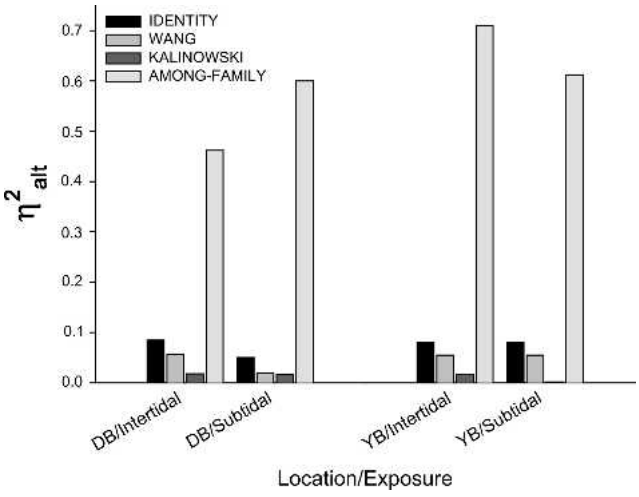

Figure 4. Strength of relatedness effect (η^2_{alt}) at each location/exposure combination. DB = Dabob Bay; YB = Yaquina Bay.

This finding is consistent with previous studies of inbreeding depression in Pacific oysters (Beattie et al. 1987, Bucklin 2002, Evans et al. 2004, Imai & Sakai 1961, Lannan 1980, Launey & Hedgecock 2001), but extends these studies to much lower levels of consanguinity among parents. Furthermore, our data indicate that the strength of inbreeding effects may vary across environments. ***I*** and ***W*** had slightly stronger associations with survival (Fig. 4) and substantially higher regression coefficients on survival at the Dabob Bay intertidal site than at other sites (Table 6), and these effects represented a much larger fraction of the among-family variance because at this site among family variance was lowest (Fig. 5). The interplay between environmental conditions and inbreeding is generally not well understood, but stressful conditions have long been believed to magnify inbreeding depression (Wright 1922). This is not, however, a universal pattern. Some studies find increased inbreeding depression in stressful environments but others do not (for reviews see Hedrick & Kalinowski 2000, Keller & Waller 2002). If overall survival can be considered an indicator of stress, our data support the hypothesis that inbreeding depression in Pacific oysters is more severe in a more stressful environment. We found the strongest effects of inbreeding in at the site with the lowest survival: Dabob Bay intertidal. Whereas only four data points for each relatedness measure precludes rigorous statistical tests, there are clear patterns for ***I*** and ***W*** of decreasing η^2_{family} with increasing survival (Fig. 6).

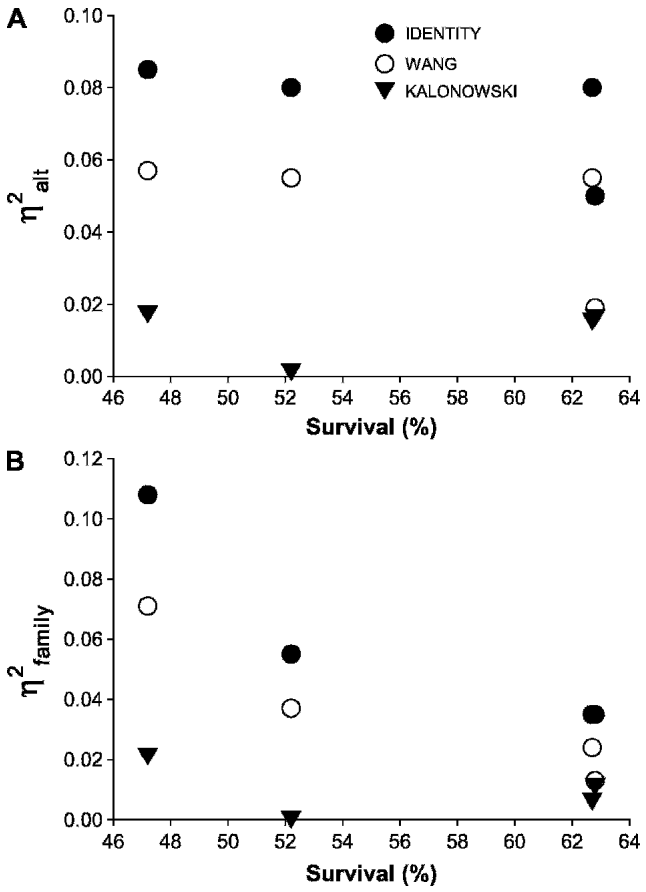

Figure 6. Scatter plots showing the relationships of a) η^2_{alt} and b) η^2_{family} with overall survival at the four test sites for each of the three relatedness estimators.

It is also important to note that whereas this study focuses on the survival of postmetamorphosis juveniles and adults, under natural conditions, larval mortality can be extremely high, is at least partially under genetic control (Ernande et al. 2003), and is impacted by inbreeding depression (Taris et al. 2007). As a consequence, this study likely underestimates the impacts of inbreeding on lifetime fitness.

Whereas we intended to also evaluate the effects of inbreeding on growth, this was impossible because of our finding that variation in stocking density resulting from differential survival confounded density-dependent environmental and genetic effects on growth. Although this is a frustrating result in the current context, we report it as a cautionary tale. Selective breeding and crossbreeding of oysters are still in their infancies, and the goals of genetic improvement and the approaches used to achieve them are still under development. A number of genetic improvement programs for bivalves evaluate families for cross-breeding or among-family selection by rearing them in separate growing units (e.g., Appleyard & Ward 2006, Langdon et al. 2003, N. G. King, L. Degremont, D. Hedgecock pers. comm.). This practice should probably be re-evaluated. Unless husbandry practices that eliminate density effects can be developed and implemented, differential survival among families is likely to produce indirect, density-mediated effects on growth as we found here, and the efficacy of genetic improvement efforts to improve growth using family-specific growout units will be compromised. Furthermore, if the goal of selection is to increase survival and growth simultaneously (e.g., by selecting for yield), density-mediated environmental correlations between these two traits could introduce severe complications. Alternative selection schemes such as within-family selection or mixed-family rearing for evaluation could minimize these problems.

Newkirk (1978), pointed out nearly three decades ago that common hatchery practices in bivalve aquaculture can result in the rapid accumulation of inbreeding and advocated simple procedures such as controlled rather than mass spawning, the maintenance of pedigree records, and separate rearing of families to minimize its effects, and others have since echoed these recommendations (e.g., Gaffney et al. 1992, Hadley 1993). Many hatcheries and breeding programs have, no doubt, instituted these or similar procedures since that time, but even the most rigorous pedigree records cannot account for the presence of relatives in the founder population. Our data indicate that kinship estimates based on molecular markers can potentially be used to improve on these procedures in at least three ways:

First, when establishing a new, reproductively closed broodstock population from a wild population, the desire to maintain a pedigreed broodstock population often imposes practical limits on the number of parents that can be used, because this requires family-specific rearing. Molecular-marker based estimates of relatedness could be used to assemble a founder population within these limitations that minimizes relatedness among individuals, maximizes genetic diversity, and minimizes inbreeding even if the natural population has substantial levels of family structure. This could be implemented by estimating the average pair-wise relatedness between each potential founder individual and all other potential founders and giving priority to individuals with the lowest overall relatedness or "mean kinship" to the rest of the population (Ballou & Lacy 1995, Doyle et al. 2001, Sekino et al. 2004). In this way, one could avoid the consequences of inbreeding among founders demonstrated in this study.

Similarly, in an already-established but unpedigreed broodstock population, mean kinship estimates on potential breeders could be either used as a criterion for maintaining genetic diversity (Doyle et al. 2001) or even incorporated into a selective breeding scheme as a component of a multitrait selection index to incorporate the retention of genetic variation into the selection goal. The advantage of such a scheme would be that phenotypically superior individuals that also have low mean kinship are likely to be carrying rare but beneficial alleles that the breeder would rather not lose from the population, and thus be given higher priority for inclusion in the broodstock population through the incorporation of mean kinship in the selection index.

Finally, even if only a limited number of founder animals are available and discarding animals with high mean kinship is not an option or a reproductively closed broodstock population already suffers from low genetic diversity, molecular marker based relatedness estimates could be used to design mating schemes that minimize the consanguinity of parental pairs used to produce either production-level spawns or the next generation for selective breeding. Whereas this approach could not

alter the fact that the founder population may be less genetically diverse than desired, it could provide immediate benefits in terms of survival by avoiding consanguineous matings and their phenotypic consequences.

ACKNOWLEDGMENTS

The authors acknowledge the contributions of Sean Matson, Drew Mosher, John Brake, and Ebru Onal in the hatchery and Alan Barton, Chris Emerson and Dan Troop in the field. Jim Krenz and Sean Matson did the DNA extractions and microsatellite genotyping. Support for this research was supplied in part from the USDA-CSREES Molluscan Broodstock Program, 2003 Mamie Markham Award and the Walter Jones Scholarship, and USDA ARS funding to the Shellfish Genetics Program (CRIS Project #5358-31000-001-00D). Three anonymous reviewers made helpful comments that greatly improved this paper. The authors also thank Oregon Oyster Farm and Taylor Shellfish Farms for the generous use of their facilities.

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